TITLE OF THE INVENTION:
FUNCTIONALIZED POLYMERIC COLLOIDS
SPECIFICATION

BACKGROUND OF THE INVENTION

1. FIELD OF INVENTION

This invention relates to polymeric colloids and methods for making them.

2. DESCRIPTION OF RELATED ART

Colloids and nanoparticles (NPs) have been widely studied for drug delivery and tissue imaging applications and recently functionalized silica nanoparticles have been explored for the modification of surfaces (1). NPs are often derived from poly (dl-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), and poly(ε-caprolactone) (PCL) due to their biocompatibility and biodegradability. When targeting of colloids (or NPs) to cells or surfaces is desired, presentation of information on the surface of the particle is critical. Therefore, it is valuable to develop means to achieve functionalization of colloids and/or NPs.

Polymeric colloids and/or NPs are typically prepared by one of the three methods.

In the method of emulsification-solvent evaporation, the polymer is dissolved in chlorinated hydrocarbon (organic solvent) such as methylene chloride or chloroform (2). The polymer solution is then mechanically dispersed in an aqueous solution containing a polymeric surfactant, such as polyvinyl alcohol (PVA) or carboxymethoxycellulose (CMC), by homogenization or ultrasonication to form a microemulsion. The thermodynamically unstable microemulsion is stabilized by the presence of PVA. The organic solvent is then evaporated and the colloids (and/or NPs) collected by centrifugation to remove the excess PVA and then resuspended in a solution of interest.

Niwa et al. (3) have developed a method to produce NPs of polymers by first dissolving the polymer in a mixture of chlorinated hydrocarbon such as methylene chloride and acetone, and then pouring this solution into a aqueous phase containing PVA with mechanical stirring. Acetone is added to enhance the diffusion of the methylene chloride solvent into the water phase. Like the solvent evaporation approach the organic solvent is evaporated and the colloids (NPs) are separated from the PVA phase by centrifugation. Their approach is called spontaneous emulsification solvent diffusion (SESD).

Murakami et al. (4) have reported a modification of the SESD procedure that relies on the gelation of the PVA phase around the emulsion droplets for stabilization of the colloids as they form in solution. In this approach, to control and restrict the gelation of PVA to the surface

of the emulsion droplet, alcohol (ethanol or methanol), which is a solvent for PVA but a non-solvent for the polymer was used. The mechanism of colloid formation is again dependent on the presence of the polymeric emulsifier, PVA. This method yields colloids of mean diameter of above 260 nm.

Despite the foregoing developments, there is still a need in the art for alternative means for providing polymeric colloids and NPs.

All references cited herein are incorporated herein by reference in their entireties.

BRIEF SUMMARY OF THE INVENTION

Accordingly, the invention provides a process for providing a polymeric colloid, said process comprising:

dissolving a polymer in a first solvent to form a first solution;

adding a second solvent to the first solution to form a second solution;

adding a third solvent to the second solution to provide the polymeric colloid,

wherein: (a) the first, second and third solvents have Drago polarities differing by less than 0.2; (b) the second solvent is miscible with the third solvent; and (c) the third solvent predominantly comprises water.

In certain embodiments, the polymer comprises at least one member selected from the group consisting of poly (dl-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL).

In certain embodiments, the first solvent is tetrahydrofuran (THF) or N-methyl-2-pyrrolidone (NMP).

In certain embodiments, the second solvent alters a polarity of the first solution.

In certain embodiments, the second solvent is acetone.

In certain embodiments, the polymer comprises at least one member selected from the group consisting of poly (dl-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL), and the first solvent is tetrahydrofuran (THF) or N-methyl-2-pyrrolidone (NMP).

In certain embodiments, the third solvent consists essentially of water.

In certain embodiments, the first solvent has a first Drago polarity of 0.80-0.99, the second solvent has a second Drago polarity of 0.80-0.99 and the third solvent has a third Drago polarity of 0.80-0.99.

In certain embodiments, the second solution is a miscible single-phase system.

In certain embodiments, the process is conducted without an emulsifying agent, a stabilizing agent and mechanical emulsification.

In certain embodiments, the polymeric colloid comprises nanoparticles.

In certain embodiments, at least about 70 wt.% of the polymer is converted to particles of the polymeric colloid.

In certain embodiments, a size of the particles is a function of a viscosity, a concentration and a polarity of at least one of the first, second and third solvents.

In certain embodiments, the process further comprises removing at least a portion of the solvents from the polymeric colloid under reduced vapor pressure.

In certain embodiments, the process further comprises adding at least one additional polymer to the second solution along with the third solvent, such that the polymeric colloid possesses a property of the at least one additional polymer. In certain of these embodiments, the at least one additional polymer is at least one member selected from the group consisting of (poly(styrenesulfonate), poly(acrylic acid sodium salt), poly(allylamine), poly(L-lysine-HCl), heparin sulfate, sulfated proteoglycans, collagen, alginic acid sodium salt and hyaluronic acid.

Also provided is a polymeric colloid provided by the process of the invention.

In certain embodiments, the polymeric colloid comprises a plurality of particles having a mean diameter of about 0.001 nm to about 1000 nm.

In certain embodiments, the polymeric colloid comprises a plurality of particles containing a composite of: (a) a first component derived from the polymer in the first solvent; and (b) a second component derived from a second polymer added to the second solution along with the third solvent.

In certain embodiments, the second component is derived from a second polymer selected from the group consisting of (poly(styrenesulfonate), poly(acrylic acid sodium salt), poly(allylamine), poly(L-lysine-HCl), heparin sulfate, sulfated proteoglycans, collagen, alginic acid sodium salt and hyaluronic acid.

In certain embodiments, the first component is derived from poly (dl-lactide-co-glycolide) and the second component is derived from (poly(styrenesulfonate), poly(acrylic acid sodium salt), poly(L-lysine-HCl) or heparin.

In certain embodiments, the particles have a mean diameter of 200 nm to 500 nm.

In certain embodiments, the particles have a zeta potential different from a reference zeta potential of a reference particle consisting essentially of poly (dl-lactide-co-glycolide).

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

The invention will be described in conjunction with the following drawings in which like reference numerals designate like elements and wherein:

Figs. 1A and 1B show schematic representations of colloid formation via the three-component approach, wherein Fig. 1A describes the relationship between the three solvent components in terms of polarity and miscibility and Fig. 2B describes the sequence of events;

Fig. 2 is a graph of colloid size as a function of viscosity of the water phase, showing that the viscosity of the water phase was increased by the addition of glycerol;

Fig. 3 is a graph of colloid size as a function of acetone (S_c) fraction, wherein the total volume is 2 ml υWater = 0.5 (i.e., in 2 ml of total formulation 1 ml is water), the remaining 1 ml is composed of THF and acetone, and υAcetone is the fraction of acetone in this 1 ml;

Fig. 4 is a graph showing the effect of aging on evolution of colloid size, wherein the asterisk represents a PDI of < 0.005;

Fig. 5 is a graph of colloid size as a function of PLGA concentration;

Fig. 6 is a graph of colloid surface charge (i.e., zeta potential) as a function of pH, for colloids prepared using Formulation 1 described below in Table 2;

Fig. 7 is a graph of colloid surface charge (i.e., zeta potential) as a function of pH, for colloids prepared using Formulation 2 described below in Table 2;

Fig. 8 is a graph showing the average size of unfunctionalized and functionalized PLGA colloids prepared using Formulations 1 and 2; and

Figs. 9A, 9B and 9C are scanning electron micrographs of PLA, PLGA-PAA and PLGA-PSS nanoparticles, respectively.

DETAILED DESCRIPTION OF THE INVENTION

The term "nanoparticles" as used herein means particles having a dimension (e.g., a diameter) within the range of about 1000 nm to about 0.001 nm. The term "colloid" as used herein means a system in which finely divided particles (e.g., nanoparticles or particles of greater or lesser dimensions) are dispersed such that the particles are not easily filtered or precipitated rapidly.

We have developed an approach to produce functionalized colloids (e.g., NPs) of various degradable polymers in a single step without the need for physical dispersion or the use of polymeric surfactants.

The invention provides a method for the rapid preparation of monodispersed, functionalized polymeric particles based on a three-component system of solvent(s) of slightly

different polarities. These solvent(s) exhibit a certain degree of miscibility with each other and combinations thereof, and one of them, such as tetrahydrofuran (THF) or N-methyl-2-pyrrolidone (NMP), is also a solvent for a polymer (Table 1, Fig. 1). Optionally, a second polymer that is a polyionic functionalizable moiety may also be included in the nanoparticles. We have verified the formation of colloids by dynamic laser light scattering (DLLS) and surface charge measurement (zeta potential).

Table 1

Solvent	Polarity/Polarizability (SPP)[a]	Miscibility represented as Polarity Index
Tetrahydrofuran (THF)	0.838	4.0
Acetone	0.881	5.1
Water	0.962	9.0
N-methyl-2-pyrrolidone (NMP)	0.970	-

[a] Drago's "universal polarity scale" (5)

In the three-component solvent system, the first component, S_p, is an organic solvent (or a miscible multisolvent system) that is used to dissolve the polymer (P_1) such as PLGA or PLA. In our studies, we have used PLGA with a composition of 50/50 dl-lactic/glycolic acid with a MW 30,000 and PLA with a MW 70,000. The second component, S_c, is a solvent that is used to alter the polarity of the polymer solution (i.e., the thermodynamic activity of both the P_1 and S_p). This S_c component is miscible with the third component, which is a water rich phase (W). The W component serves as the solvent for the polyionic functionalizable polymeric moiety (P_2). Two non-limiting examples of the three-component solvent systems are shown in Table 2 to illustrate the method of this invention.

Table 2

Formulation	S_p	S_c	W
1	THF (0.125)	Acetone (0.375)	Water (0.500)
2	NMP (0.375)	Acetone (0.125)	Water (0.500)

Values in parentheses indicate volume fraction in the formulation

THF and NMP are preferred polymer solvents due to their ability to dissolve a wide range of polymers. Thus far, we have obtained monodispersed PLGA and PLA colloids (NPs) as small as 192 nm and 150 nm using Formulations 1 and 2, respectively.

In a preferred embodiment of the inventive process for preparing particles of the invention, the first step is the dissolution of polymer(s) P_1 in desired solvent(s), S_p. To this (S_p), S_c is added to form a miscible single-phase system. The formation of colloids is then induced by the addition of a water-rich phase (W) with or without P_2. The organic solvent is then removed under reduced pressure. Since THF has a high vapor pressure, it can be easily removed, which is a desirable attribute for biomedical applications of NPs. NMP has a low vapor pressure, but its safety profile is more favorable and the residual NMP concentration in our study is still acceptable. The colloidal suspension is concentrated by a factor of 2-3 depending on the system by removing the volatile phases without any appreciable change to both the size and the polydispersity index (PDI) of the colloid. This is indicative of the fact that the colloids do not aggregate and are rather stable.

The rationale behind the choice of three-component system with slightly differing polarities is to tailor the thermodynamic activity of the polymer so as to achieve uniform and spontaneous nucleation, growth and solidification of the polymer NP phase, resulting in a colloidal suspension that is stable without the need to add an emulsifying or stabilizing agent such as PVA. The proposed mechanism of nanoparticle formation is as follows (Fig. 1): When S_c is added to the polymer solution composed of P_1 and S_p, the composition of the solvation shell around the polymer is altered to include both S_p and S_c. Upon addition of W, the solvation shell around the polymer is further altered and enriched in water. Since W is a non-solvent for the polymer, this results in a precipitation and solidification of the polymer phase. The miscibility of W with Sp and Sc ensures the process occurs uniformly and often rapidly in the entire reactor volume, resulting in a large population of mono-dispersed polymeric colloids with a typical yield of 80% with respect to P_1 weight. Preferably, the yield is at least 50 wt.%, more preferably at least 70 wt.%, even more preferably at least 90 wt.%.

The nature of the W (water) phase is an important means for the control of colloid formation and colloid size. This has been verified by two separate experiments. First, if the diffusion of W phase is important, then any change to its diffusivity will alter colloid size. We have observed that increasing the viscosity of the W phase, with the addition of glycerol to W, which diminishes W diffusivity, results in an increase in the colloid size (Fig. 2). Second, increasing the affinity of water to the polymer solvation core should diminish the colloid size due to enhanced tendency for precipitation. When the ratio of THF:Acetone is diminished, thereby increasing the polarity of the system, which should increase the affinity for W, the colloid size indeed decreases as predicted. By varying the volumetric ratio of S_p to S_c in the

THF:Acetone:Water system, we found a positive linear correlation between acetone volume fraction ($\upsilon_{acetone}$) and colloid size, with an R^2 value of 0.9986 (Fig. 3). Here,

$$\upsilon_{Acetone} = V_{Acetone}/(V_{Acetone} + V_{THF}) \quad \text{Eq. 1}$$

The importance of the polarity difference between W phase and Sp/Sc in colloid formation is further illustrated by the following observations (Fig. 4).

Mean colloid size in NMP:Acetone:Water system is much smaller compared to THF:Acetone:Water system. This is because the polarity of W is much closer to that of NMP than to THF.

In the NMP:Acetone:Water system no appreciable change in colloids size is observed (no change in polydispersity index (PDI)) as a function of time and agitation, which is suggestive of fast and rapid polymer solidification kinetics. This is consistent with the close polarity of NMP and water (Table 1). However, in the THF:Acetone:Water system the colloids size decreases (decrease in PDI) as a function of time and agitation suggestive of slower polymer solidification kinetics, which is consistent with the differences in polarity between THF and water (Table 1).

We have also shown that colloids of various sizes can be prepared by increasing the concentration of the polymer in S_p (Fig. 5). Larger colloids form when the polymer concentration increases. In addition, temperature may be used to influence precipitation of the polymer such as in the case of lower critical solution temperature (LCST) polymers that undergo first-order transitions such as gelation upon heating or cooling. Examples of LCST polymers include poly(isoproprylamide) (poly(IPAm)) and co-polymers thereof and copolymers of ethylene oxide.

Since the water rich phase, W, is directly involved in colloid formation, most likely at the interface of polymer and its solvation shell, it can be used to deliver polymeric species to the colloid. These polymeric species may possess functionalizable groups such as synthetic polyelectrolytes (poly(styrenesulfonate), (PSS, MW 70k); poly(acrylic acid sodium salt) (PAA, MW 2k); poly(allylamine), poly(L-lysine-HCl), (PLys, MW 22.1 k) and biological polyelectrolytes (heparin sulfate, sulfated proteoglycans, collagen) and other polymers (alginic acid sodium salt, hyaluronic acid), as long as they are soluble in water. They are collectively referred to as P_2, and they can be co-precipitated with the P_1 to yield functionalized colloids possessing P_2's functionality (charge, for example). PLGA and PLA colloids bearing PSS, PAA (negatively charged surface) and PLys (positively charged surface) moieties on the surface have been prepared using the formulations of Table 2. The presence of PSS, PAA and PLys groups on

the colloid surface was verified by the determination of surface charge as a function of pH, i.e., by measuring the zeta potential of the colloidal system (Figs. 6 & 7). The surface charge of PLGA, PLGA-PAA and PLGA-PSS colloids at neutral pH are all negative, so the negative groups (PAA and PSS) do not have a large effect on the surface charge of PLGA. However, when a positive group (PLys) is used, the surface charge of PLGA/PLys becomes positive at neutral pH, indicating that the colloids do indeed possess the positive group on their surface. The functionalization of colloids with polyions (polyelectrolytes) typically results in a negligible to moderate increase in colloids size depending on the system employed (Fig. 8).

Table 3 describes the change in morphology of nanoparticles that is observed under different polyelectrolyte loading conditions and various solvent systems.

Table 3
Nanoparticle size and polydispersity index for nanoparticle produced using various solvent systems

Polymer	Polyelectrolyte	Solvent System	Size	PDI
PLGA	None	THF/Acetone	243	0.05
PLGA	PAA	THF/Acetone	271	0.07
PLGA	PSS	THF/Acetone	404	0.09
PLGA	PLys	THF/Acetone	259	0.07
PLA	None	Dichloromethane/ Acetone	184	0.08

Table 4 confirms the presence of each polyelectrolyte on the surface of functional nanoparticles. More specifically, Table 4 shows the pK_a of the functional group of each nanoparticle, and its corresponding measured isoelectric point and zeta potential at physiological pH 7.4.

Table 4

Nanoparticle Composition	pK_a of Functional Group	pI_e of Nanoparticle Surface	$\Delta\zeta$ from PLGA	ζ at pH 7.4
PLGA	--	2.75	0	-26.7
PLGA-PSS	~2	2.40	-0.35	-28.3
PLGA-PAA	~3.5	2.80	+0.05	-26.2
PLGA-PLys	~10	9.50	+6.75	16.9
PLGA-Hep	2-4	3.40	+0.65	-28.1
PLGA-PEG	--	N/A	--	-28.3

The inventive process is significantly different from the solvent evaporation approach and the SESD methods in the following ways. First, we have eliminated the need to use emulsifying agents or surfactants, or gelation agents, such as PVA. Therefore, the issue of biocompatibility associated with such agents is also eliminated. The polymer colloids precipitated in our method are stable in the three-component solvent we use and do not require additional stabilization agent at the colloid-solvent interface. Second, we have eliminated the need for mechanical emulsification (sonication) (6) to create the particle dispersion. Our choice of three-component solvent phases that are highly miscible based on their polarities makes it possible to have uniform precipitation without mechanical emulsification. Third, our method prepares in one step colloids that may exhibit different functionality, by varying the polyionic species incorporated in the W phase. The colloids prepared have two components, one from the polymer (P_1) initially dissolved in the Sp phase, and the other from the polymeric polyionic species (P_2) initially dissolved (dispersed) in the W phase. Such composite colloids are not usually possible in the prior art.

The colloids prepared by the inventive process are naturally stable as they are the thermodynamically favored end point, achieved by the judicious choice of solvents with slightly differing polarities.

Functionalized colloids described herein, with the appropriate further surface and bulk modification (such as introduction of magnetic species) can be used as tissue and cell imaging agents and in cell separation technologies. Both the polymer in the S_p phase and the polyionic polymer in the W phase can be varied so to suit various applications. The precipitation of colloids using our three-component solvent systems can also be carried out at the same time as shape processing. For example, the solvent can be spin coated, extruded, or poured when precipitation occurs at the same time or after such operation. Additional functionality and information can be achieved by well known electrostatic layer-by-layer assembly methodologies (7) and by covalent modification of functionalizable moieties such as amine, carboxylic acid etc., with ligands and biomolecules of choice. The functionalized or modified colloids can be used to impart functionality to hard materials such as metals and ceramics and soft materials such as polymers (synthetic and biological), by either dispersion in the bulk or as surface coatings. The functionalized colloids may be co-extruded, co-spun, co-gelled, co-precipitated and coacervated with hard and soft materials of choice. The functionalized colloids can also be provided as coatings on fibers (woven and non-woven), felts, sheets, films and other solid objects such as metallic and polymer-derived or polymer-based implants and devices. Such

functionalized colloids can also be used in drug delivery. A biologically active agent, such as drugs, peptides, proteins, DNA or DNA-snipets, can be introduced within the colloid or on the surface (e.g., DNA assembly on positively charged colloids) and then administered via oral, injectable, topical or pulmonary routes.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

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